MR 446915

Figure 1

Activation of the Transcription Factor NF-κB through TNF Receptor 2 in CT6 Cells

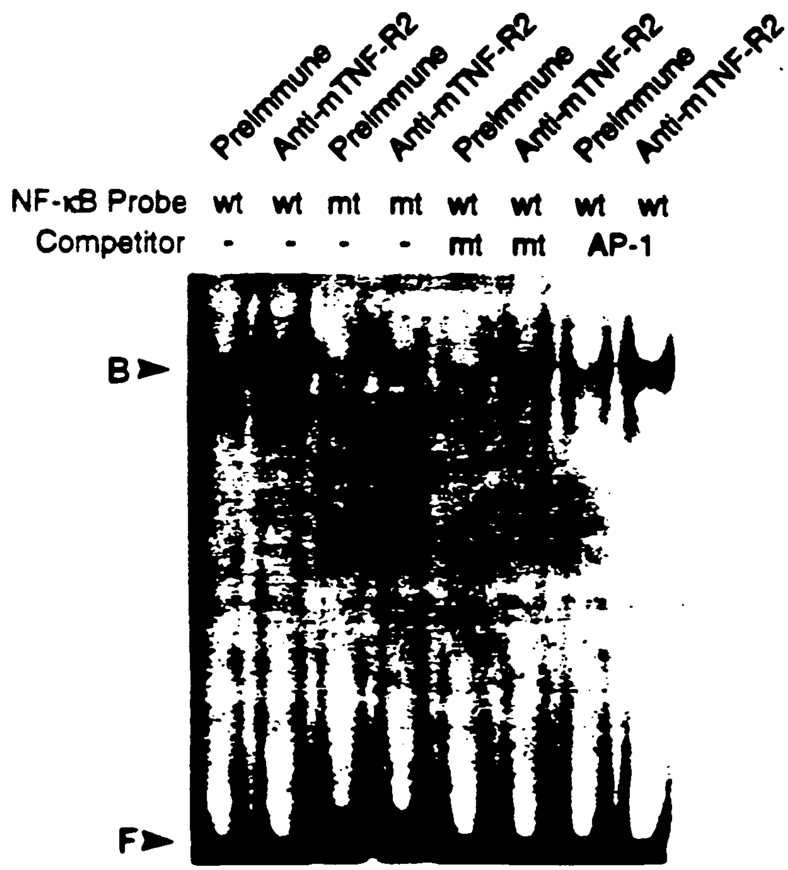

Figure 2

Immunoprecipitation of Human TNF Receptor 2

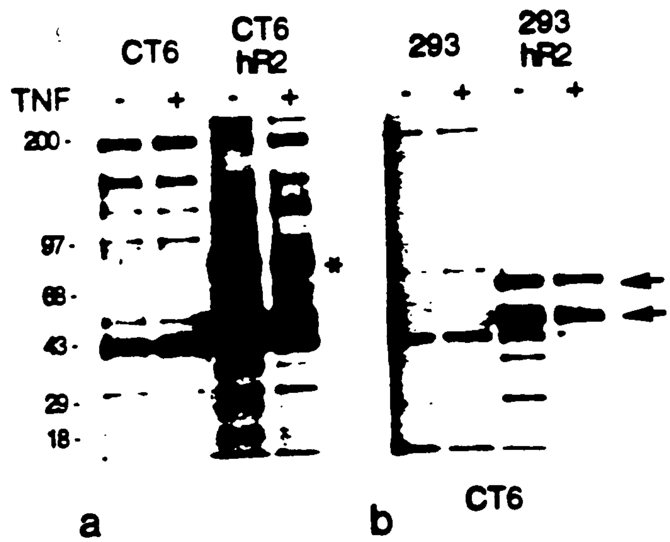

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Figure 3

Glutathione-S-Transferase human TNF Receptor 2
Intracellular Domain Fusion Protein

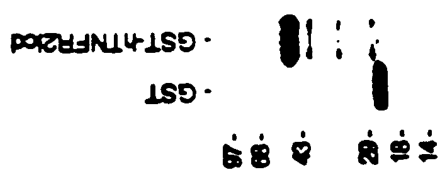

Figure 4

Coprecipitation of Glutathione-S-Transferase
Human TNF Receptor 2 Intracellular Domain
Fusion Protein in CT6 Cell Extracts

Figure 5

Coprecipitation of Glutathione-S-Transferase Mutant
Human TNF Receptor 2 Intracellular Domain
Fusion Proteins in CT6 Cell Extracts

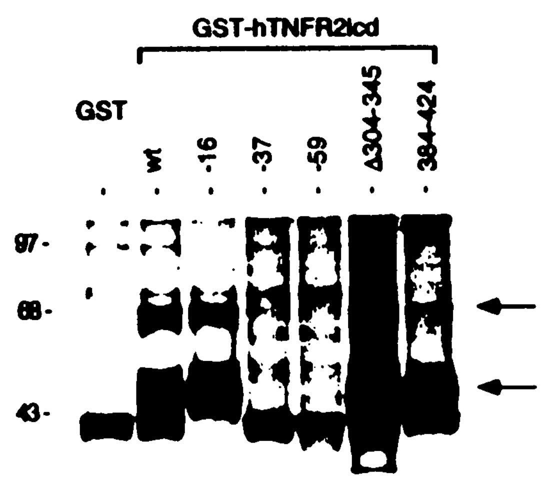

Figure 6

Competition of TNF Receptor 2 Associated Factors with Glutathione-S-Transferase TNF Receptor 2 Intracellular Domain Fusion Proteins

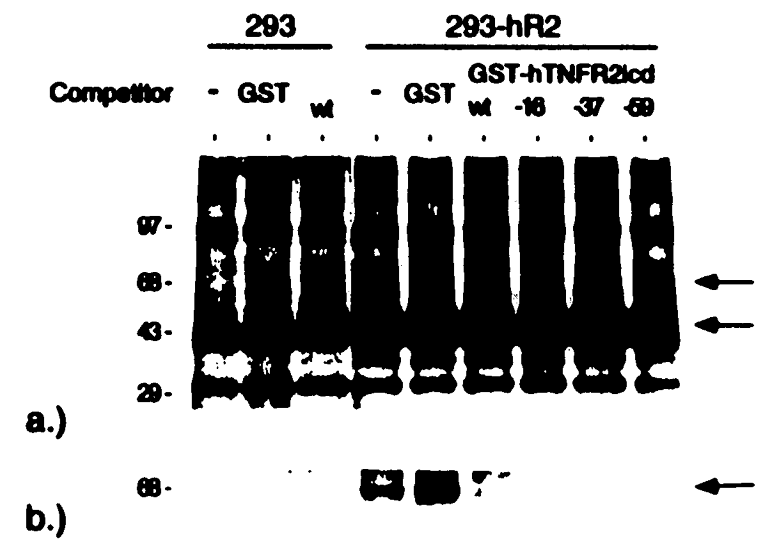

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Figure 7

Coprecipitation of Glutathione-S-Transferase Human TNF Receptor 2 Intracellular Domain Fusion Protein in Jurkat Cell Extracts

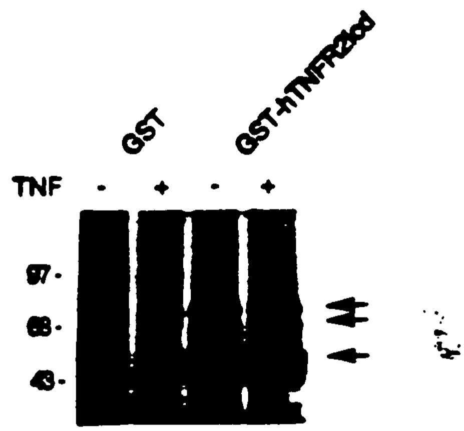

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Figure 8

Intracellular Localization of TNF Receptor 2 Associated Factors

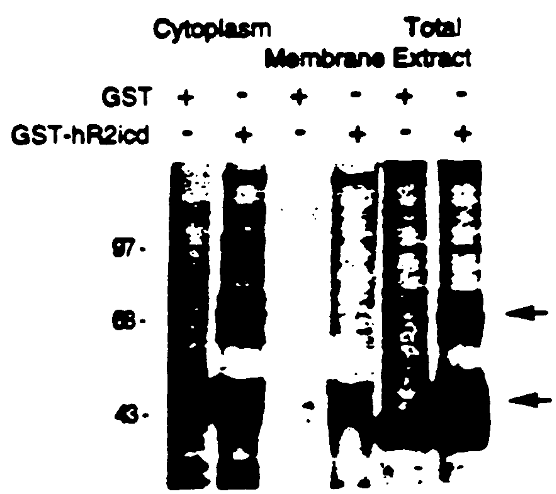
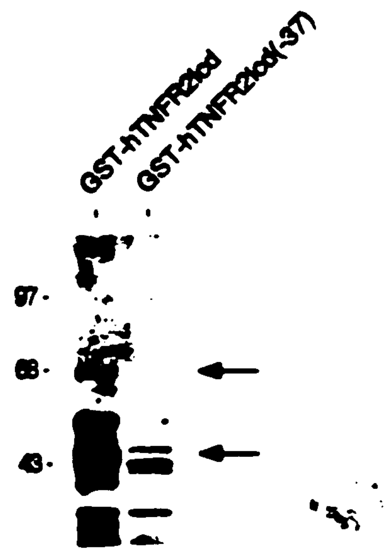


Figure 9

Purification of TNF Receptor 2 Associated Factors



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149   GCCACCCAGATATCCTGGAGGACCCAGAACCCTGGAGATTCCCATCAGAAAGACCTTCTGGCCACCTGAAACCCC

1       MetAlaSerSerSerAlaProAspGluAsnGluPheGlnPheGlyCysProProAlaProCysGlnAspPro
224   AAGATGGCCTCCAGCTCAGCCCCTGATGAAAACGAGTTTCAATTTGGTTGCCCCCCTGCTCCCTGCCAGGACCCA

25    SerGluProArgValLeuCysCysThrAlaCysLeuSerGluAsnLeuArgAspAspGluAspArgIleCysPro
299   TCGGAGCCCAGAGTTCTCTGCTGCACAGCCTGTCTCTCTGAGAACCTGAGAGATGATGAGGATCGGATCTGTCCT

50    LysCysArgAlaAspAsnLeuHisProValSerProGlySerProLeuThrGlnGluLysValHisSerAspVal
374   AAATGCAGAGCAGACAACCTCCATCCTGTGAGCCCAGGAAGCCCTCTGACTCAGGAGAAGGTTCACTCTGATGTA

75    AlaGluAlaGluIleMetCysProPheAlaGlyValGlyCysSerPheLysGlySerProGlnSerMetGlnGlu
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100   HisGluAlaThrSerGlnSerSerHisLeuTyrLeuLeuLeuAlaValLeuLysGluTrpLysSerSerProGly
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125   SerAsnLeuGlySerAlaProMetAlaLeuGluArgAsnLeuSerGluLeuGlnLeuGlnAlaAlaValGluAla
599   TCCAACCTAGGGTCTGCACCCATGGCACTGGAGCGGAACCTGTCAGAGCTGCAGCTTCAGGCAGCTGTGGAAGCG

150   ThrGlyAspLeuGluValAspCysTyrArgAlaProCysCysGluSerGlnGluGluLeuAlaLeuGlnHisLeu
674   ACAGGGGACCTGGAGGTAGACTGCTACCGGGCACCTTGCTGTGAGAGCCAGGAAGAACTGGCCCTGCAGCACTTG

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749   GTGAAGGAGAAGCTGCTGGCTCAGCTGGAGGAGAAGCTGCGTGTGTTTGCAAACATTGTTGCTGTCCTCAACAAG

200   GluValGluAlaSerHisLeuAlaLeuAlaAlaSerIleHisGlnSerGlnLeuAspArgGluHisLeuLeuSer
824   GAAGTGGAGGCTTCCCACCTGGCACTGGCCGCCTCCATCCACCAGAGCCAGTTGGACCGAGAGCACCTCCTGAGC

225   LeuGluGlnArgValValGluLeuGlnGlnThrLeuAlaGlnLysAspGlnValLeuGlyLysLeuGluHisSer
899   TTGGAGCAGAGGGTGGTGGAATTACAGCAAACCCTGGCTCAAAAAGACCAGGTCCTGGGCAAGCTTGAGCACAGT

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974   CTGCGACTCATGGAGGAGGCATCCTTTGATGGTACTTTCCTGTGGAAGATCACCAATGTCACCAAGCGGTGCCAC

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300   LeuArgLeuTyrLeuAsnGlyAspGlySerGlyLysLysThrHisLeuSerLeuPheIleValIleMetArgGly
1124  CTGCGCTTGTACCTGAACGGGGATGGCTCAGGCAAGAAGACCCACCTGTCCCTCTTCATCGTGATCATGAGAGGA

325   GluTyrAspAlaLeuLeuProTrpProPheArgAsnLysValThrPheMetLeuLeuAspGlnAsnAsnArgGlu
1199  GAATACGATGCTCTCCTGCCCTGGCCTTTCAGGAACAAGGTCACCTTTATGCTACTTGACCAGAACAACCGAGAG

350   HisAlaIleAspAlaPheArgProAspLeuSerSerAlaSerPheGlnArgProGlnSerGluThrAsnValAla
1274  CATGCTATTGATGCCTTCCGGCCTGACCTGAGCTCAGCCTCCTTCCAGCGGCCACAGAGTGAGACCAACGTGGCC

375   SerGlyCysProLeuPhePheProLeuSerLysLeuGlnSerProLysHisAlaTyrValLysAspAspThrMet
1349  AGCGGCTGCCCGCTCTTCTTCCCCCTCAGCAAGCTGCAGTCACCCAAGCACGCCTACGTCAAAGATGACACAATG

400   PheLeuLysCysIleValAspThrSerAla
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1499  TGGGGGACTTAGCTAGACAGCCAGGCCCTGCCTGCCCTTGGAGCCCACAGCCCACGACAAGGAGGAGCCAAGGCT
1574  GGCATGACTTCAGCGCCACAGCATGCTGGTTATGGCTGATGTGAGGCTGGAGAAACGTGTGCGTACAGAGACAGA
1649  GTGGAGGAGAAGACAGAAGTGCTCTTTTCACACAGACTACACGACACCAGGAGGCCAGCATGCCAGCAGCTTCTG
1724  AATGTTGAGACCAGCCTAGATCAGGATGAAAAGAGCCAGGCCTGAGGCTTGGACATTGAGCCAAGGCTATGGGGC
1799  CTAAGTGGAGGGGCACTCCTACCAGGACATTCTCTCGAGGTCAGGGCATAACTGGAAAAATGCCCCCATCTCTCT
1874  GTTCAGACTCAAAACTAGAACCACAGGGCAGAAGGGTCAGACATTAATGTGAATTTAACCTGCCCTGGACTGAGT
1949  TCCTATGTTAACAGACACGCAAACAGGTAAACCCAGAAACTGCCCTGGGAAATGCTTTCTGGCTGCATCTGGAGA
2024  TCTTTGATGTTTTTACCGACAAAACAAATAACAAAAGCCTTGAATTGCAAAAAAAAAAAAAAAAA
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Figure 10

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1
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75   GGCTCCCTAGAACTGCTACAGCCTGGCTTCTCCAAGACCCTCCTGGGGACCAGGTTAGAAGCCAAGTACCTCTGT

35   SerAlaCysLysAsnIleLeuArgArgProPheGlnAlaGlnCysGlyHisArgTyrCysSerPheCysLeuThr
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60   SerIleLeuSerSerGlyProGlnAsnCysAlaAlaCysValTyrGluGlyLeuTyrGluGluGlyIleSerIle
225  AGCATCCTCAGCTCTGGGCCCCAGAACTGTGCTGCCTGTGTCTATGAAGGCCTGTATGAAGAAGGCATTTCTATT

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375  GATGGATGCACTTGGAAGGGGACCTTGAAAGAATACGAGAGCTGCCACGAAGGACTTTGCCCATTCCTGCTGACG

135  GluCysProAlaCysLysGlyLeuValArgLeuSerGluLysGluHisHisThrGluGlnGluCysProLysArg
450  GAGTGTCCTGCATGTAAAGGCCTGGTCCGCCTCAGCGAGAAGGAGCACCACACTGAGCAGGAATGCCCCAAAAGG

160  SerLeuSerCysGlnHisCysArgAlaProCysSerHisValAspLeuGluValHisTyrGluValCysProLys
525  AGCCTGAGCTGCCAGCACTGCAGAGCACCCTGTAGCCACGTGGACCTGGAGGTACACTATGAGGTCTGCCCCAAG

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210  SerLysCysArgValLeuCysArgPheHisThrValGlyCysSerGluMetValGluThrGluAsnLeuGlnAsp
675  AGCAAATGCCGGGTTCTCTGCAGATTCCACACCGTTGGCTGTTCAGAGATGGTGGAGACTGAGAACCTGCAGGAT

235  HisGluLeuGlnArgLeuArgGluHisLeuAlaLeuLeuLeuSerSerPheLeuGluAlaGlnAlaSerProGly
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260  ThrLeuAsnGlnValGlyProGluLeuLeuGlnArgCysGlnIleLeuGluGlnLysIleAlaThrPheGluAsn
825  ACCTTGAACCAGGTGGGGCCAGAGCTACTCCAGCGGTGCCAGATTTTGGAGCAGAAGATAGCAACCTTTGAGAAC

285  IleValCysValLeuAsnArgGluValGluArgValAlaValThrAlaGluAlaCysSerArgGlnHisArgLeu
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310  AspGlnAspLysIleGluAlaLeuSerAsnLysValGlnGlnLeuGluArgSerIleGlyLeuLysAspLeuAla
975  GACCAGGACAAGATTGAGGCCCTGAGTAACAAGGTGCAACAGCTGGAGAGGAGCATCGGCCTCAAGGACCTGGCC

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410  PhePheValValMetLysGlyProAsnAspAlaLeuLeuGlnTrpProPheAsnGlnLysValThrLeuMetLeu
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435  LeuAspHisAsnAsnArgGluHisValIleAspAlaPheArgProAspValThrSerSerSerPheGlnArgPro
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460  ValSerAspMetAsnIleAlaSerGlyCysProLeuPheCysProValSerLysMetGluAlaLysAsnSerTyr
1425 GTCAGTGACATGAACATCGCCAGTGGCTGCCCCCTCTTCTGCCCTGTGTCCAAGATGGAGGCCAAGAATTCCTAT

485  ValArgAspAspAlaIlePheIleLysAlaIleValAspLeuThrGlyLeu
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2100 ACGACAAAAAAAAAAAAAAAAA
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Figure 11

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Figure 12a

A

TRAF2	(mouse)	31	KYLCSACKNILRRPFQA	QCGHRYCSFCLTSI	LSS	GPQNCAACVYE
COP1	(*A. thaliana*)	49	DLLCPICMQIIKDAFLT	ACGHSFCYMCIITH	LRN	KSDCPCCSQH
EFP	(human)	10	ELSCSICLEPFKEPVTT	PCGHNFCGSCLNETWA	VQG	SPYLCPQCRAV
RAD-18	(*S. cerevisiae*)	25	LLRCHICKDFLKVPVLT	PCGHTFCSLCIRTH	LNN	QPNCPLCLFE
UVS-2	(*N. crassa*)	31	AFRCHVCKDFYDSPMLT	SCNHTFCSLCIRRC	LSV DSK	CPLCRAT
RAG-1	(human)	290	SISCQICEHILADPVET	NCKHVFCRVCILRC	LKV	MGSYCPSCRYP
SS-A/Ro	(human)	13	EVTCPICLDPFVEPVSI	ECGHSFCQECISQV	GKG	GGSVCAVCRQR
RING1	(human)	16	ELMCPICLDMLKNTMTTKECLHRFCSDCIVTA		LRS	GNKECPTCRKK
RPT-1	(mouse)	12	EVTCPICLELLKEPVSA	DCNHSFCRACITLNYESNRNTDGKGNCPVCRVP		
RFP	(human)	13	ETTCPVCLQYFAEPMML	DCGHNICCACLARCWGTA		ETNVSCPQCRET
c-cbl	(human)	378	FQLCKICAENDKDVKIE	PCGHLMCTSCLTS	WQESEGQ	GSSGCPFCRCE

consensus ---C--C----------C-H--C--C---------------C--C---

X11-12 (between C--C and C-H); X10-16 (between C and C)

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B

Figure 12b

TRAF2	(mouse)	157	CPKRSLS**C**QH**C** RAPCSHVDLEV**H**YE V**C**
		182	PKFPLT**C**DG**C**GKKKIPRETFQD**H**VR A**C**
DG17	(*D. discoideum*)	171	GGFKLVT**C**DF**C**KRDDIKKKELET**H**YK T**C**
TFIIIA	(*X. laevis*)	189	QD LAV**C**DV**C**NRKFRHKDYLRD**H**QK T**H**
XLCOF14	(*X. laevis*)	1	TGKYPFI**C**SE**C**GKSFMDKRYLKI**H**SN V**H**
XFIN	(*X. laevis*)	1225	TGEKPYT**C**TV**C**GKKFIDRSSVVK**H**SR T**H**
ZFY1/2	(mouse)	521	RKKFPHI**C**GE**C**GKGFRHPSALKK**H**IR V**H**
MFG2	(mouse)	293	SEEKPFE**C**EE**C**GKKFRTARHLVK**H**QR I**H**
RAD18	(*S. cerevisiae*)	183	PNEQMAQ**C**PI**C**QQFYPLKALEKT**H**LD E**C**
UVS-2	(*N. crassa*)	182	PDDGLVA**C**PI**C**LTRM KEQQVDR**H**LDTS**C**

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TRAF2    1 MAAASVTSPGSLELLQPGFSKTLLGTRLEAKYLCSACKNILRRPFQAQCG

TRAF2   51 HRYCSFCLTSILSSGPQNCAACVYEGLVEEGISILESSSAFPDNAARREV

TRAF2  101 ESLPAVCPNDGCTWKGTLKEYESCHEGLCPFLLTECPACKGLVRLSEKEH
TRAF1    1 ...............................MASSSAPDENEFQFSCPPA

TRAF2  151 HTEQECPKRSLSCQHCRAPCSHVDLEVHYEVCPKFPLTCDGCGKKKIPRE
TRAF1   20 PCQDPSEPRVLCCTACLSENLRDDEDRICPKCRADNLHPVSPG-SPLTQE

TRAF2  201 TFQDHVRACSKCRVLCRFHTVGCSEMVETENLQDHELQRLREHLALLLSS
TRAF1   69 KVHSDV---AEAEIMCPFAGVGCSFKGSPQSMQEHEATSQSSHLYLLLAV

TRAF2  251 FLEACASPGTLNQVGPELLQR.............................
TRAF1  116 LKEWKSSPGSNLGSAPMALERNLSELQLQAAVEATGDLEVDCYRAPCCES

TRAF2  272 ................CQILEQKIATFENIVCVLNREVERVAVTAEACSRQH
TRAF1  166 CEELALQHLVKEKLLAQLEEKLRVFANIVAVLNKEVEASHLALAASIHQS

TRAF2  306 RLDQDKIEALSNKVQQLERSIGLKDLAMADLEQKVSELEVSTYDGVFIWK
TRAF1  216 CLDPEHLLSLEQRVVELCQTLAQKDCVLGKLEHSLRLMEEASFDGTFLWK

TRAF2  356 ISDFTRKRQEAVAGRTPAIFSPAFYTSRYGYKMCLRVYLNGDGTGRGTHL
TRAF1  266 ITNVTRFCHESVCGRTVSLFSPAFYTAKYGYKLCLRLYLNGDGSGKKTHL

TRAF2  406 SLFFVVMKGPNCALLQWPFNQKVTLMLLDHNNREHVIDAFRPDVTSSSFQ
TRAF1  316 SLFFILMRGEYCALLPWPFRNKVTFMLLDQNNREHAIDAFRPDLSSASFQ

TRAF2  456 RPVNDMNIASGCPLFCPVSKME-AKNSYVRDDAIFIKAIVDLTGL
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Figure 13

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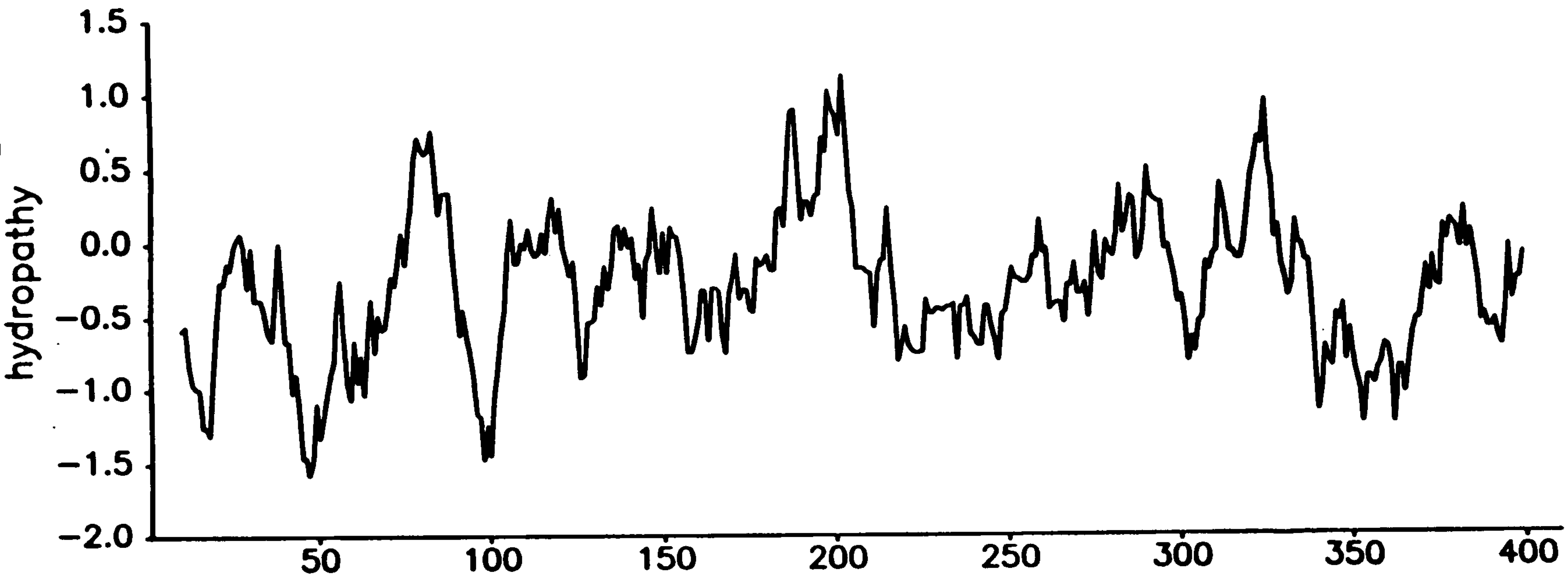

Figure 14a

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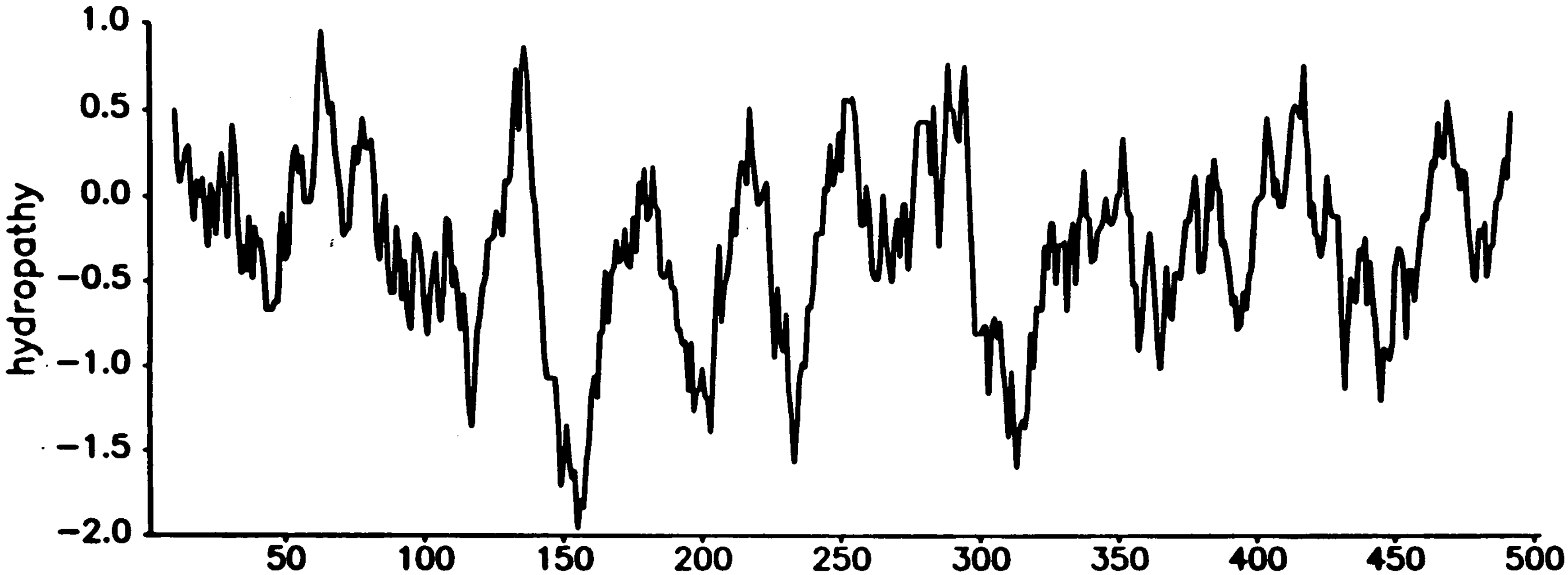

Figure 14b

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TRAF Expression in CT6 Cells

Figure 15a

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Figure 15b

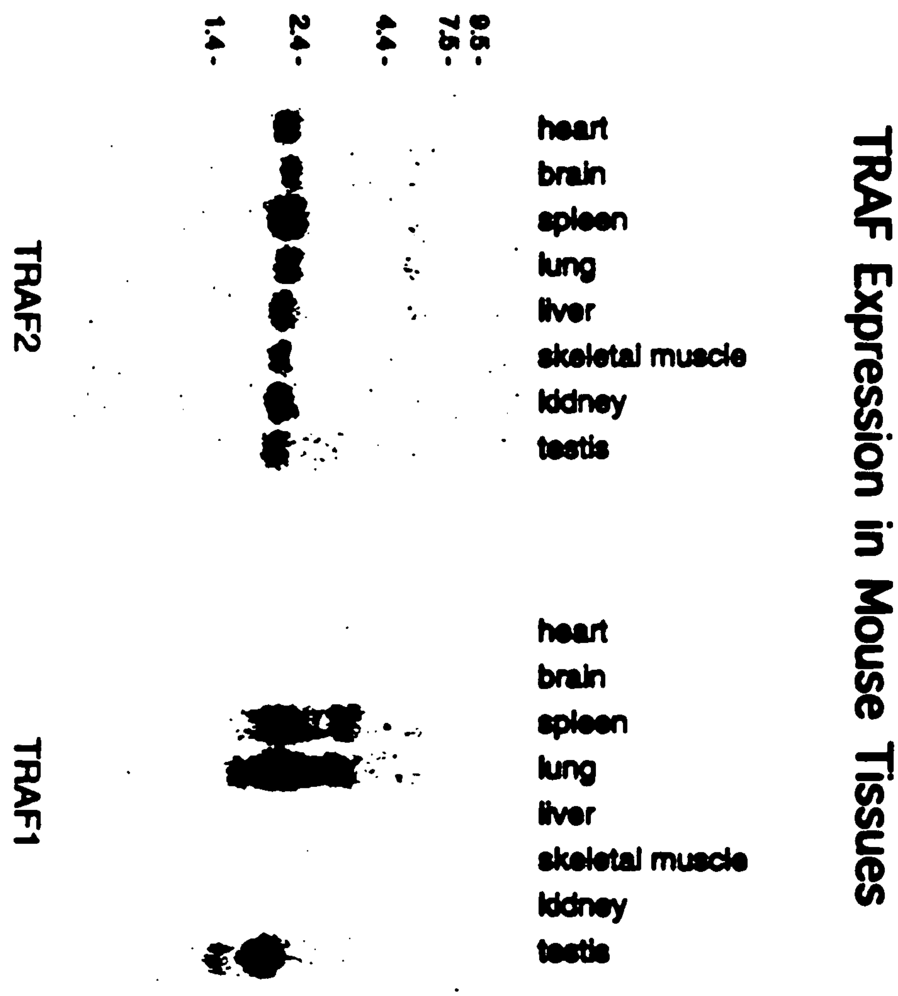
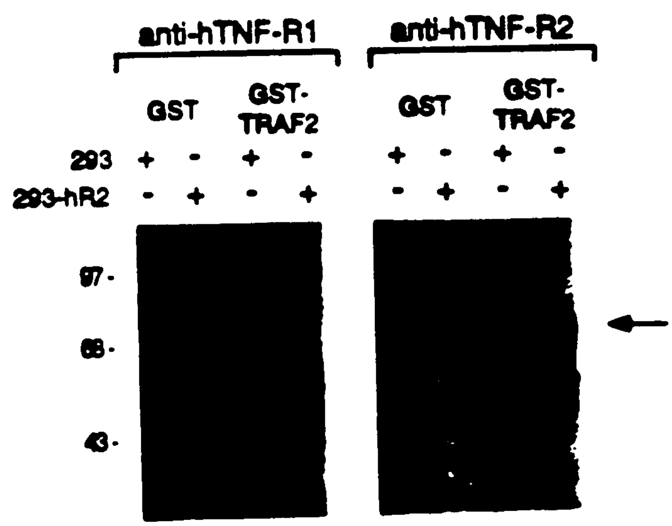
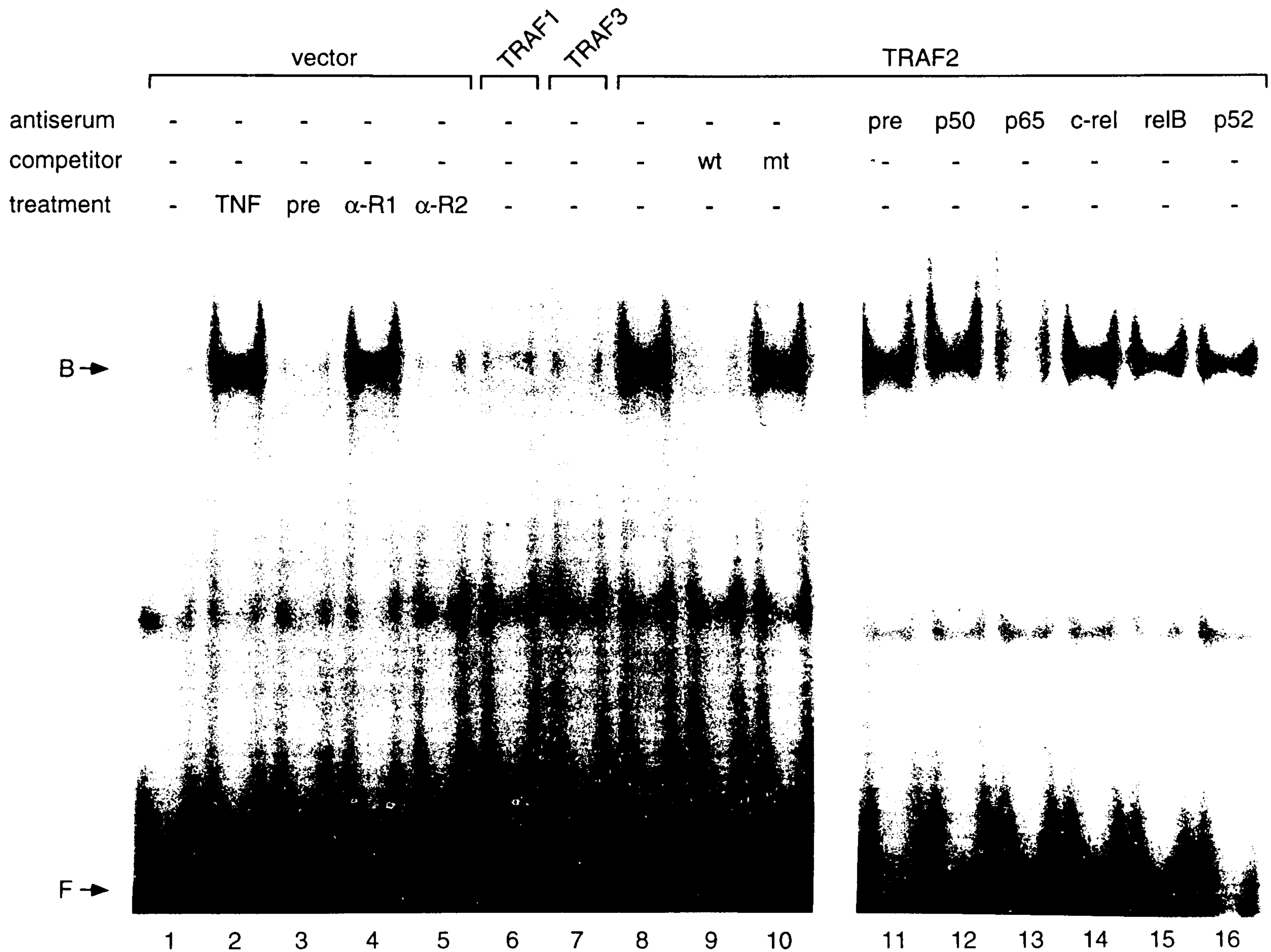


Figure 16

A Glutathione-S-Transferase TRAF2 Fusion Protein Coprecipitates the Human TNF-R2 in 293 Cell Extracts

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TRAF1
TRAF3
TRAF2
antiserum - - - - - - - - - - pre p50 p65 c-rel relB p52
competitor - - - - - - - - wt mt - - - - - -
treatment - TNF pre α-R1 α-R2 - - - - - - - - - - -
B
F
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure 17

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Figure 18

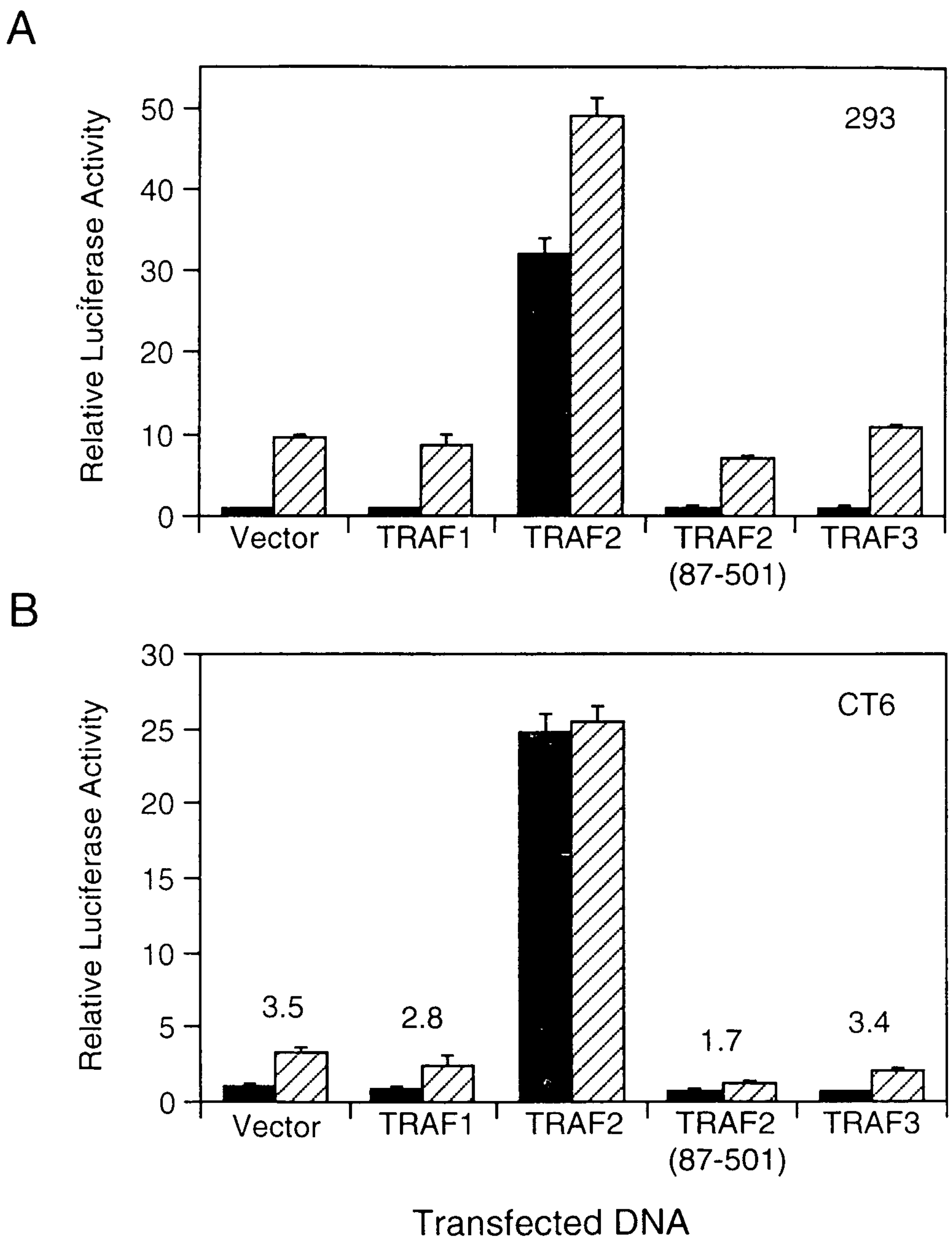

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Figure 19